Hangzhou Simgen Biotechnology Co., Ltd.
5th floor, Building No. 4, No. 8 Xiyuan 1st Road, Hangzhou, P. R. China.
Pc: 310030 Tel: +86-571-87381295 Fax: +86-571-87381295

DNA/RNA Parallel Extraction Kit Instructions

Composition

DNA/RNA Parallel Extraction Kit Cat. No.	5 Preps 5002005	50 Preps 5002050
DNA Spin Columns	5	50
RNA Spin Columns	5	50
β-mercaptoethanol	50 μl	500 μl
Buffer RLT	4 ml	32 ml
Buffer WA	3 ml	24 ml
Buffer WBR	2 ml	19 ml
RNase-Free Water	1.5 ml	2 ml×3
Instructions	1	1

Storage

1. All the reagents and components can be stored for up to 2 years at room temperature (0~30℃). For longer storage, it is recommended to keep at 2-8℃.
2. The product stored at 2~8℃ should be restored to room temperature before use.

Technical Support

R&D Department, Hangzhou Simgen Biotechnology Co., Ltd. E-mail: technical@simgen.cn , Tel: 400-0099-857.

Introduction

This product is no need of phenol chloroform extraction and is suitable for extracting DNA and RNA from ≤ 25 mg tissue or ≤1×10^7 cultured cells. The samples were lysed in the lysis buffer and filtered through the DNA and RNA Spin Columns, respectively, and the solubilized proteins and PCR inhibitors were filtered out. Finally, the DNA or RNA bound to the Spin Column is eluted by RNase-Free Water, which can be used for various molecular biology experiments such as PCR, RT-PCR, Northern blot, Dot blot, mRNA isolation, etc.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol and 70% ethanol.
2. RNase-free 1.5 ml centrifuge tubes.
3. Pipettes and tips (RNase-free pipette tips with filters are recommended to avoid RNase contamination).
4. Disposable gloves and protective equipment and tissues.
5. Microcentrifuge(s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
6. Vortexer.
7. RNase-free use labs.

Preparation Before Use

1. If the centrifuge has refrigeration function, set the temperature to 25℃.
2. Add 10 μl β-mercaptoethanol per 1 ml Buffer RLT and mix well. The Buffer RLT with β-mercaptoethanol for one month did not affect the experimental results.
3. Add absolute ethanol to Buffer WA and Buffer WBR according to the instructions on the label the reagent bottle and tick the box on the label to mark "Ethanol Added".
4. Since saliva and skin contain RNases, please wear latex gloves and a mask throughout the RNA extraction process.

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Protocol For Animal Tissues

1. **Weigh 200~400 mg chopped animal tissue in a mortar, grind the sample to powder form with liquid nitrogen, and then weigh 20~25 mg powdered tissue with a 1.5 ml centrifuge tube pre-cooled with liquid nitrogen.**

* When grinding the tissue, liquid nitrogen should be added in time to avoid the tissue thawing, so as to avoid the degradation of RNA due to the reactivation of endogenous RNase.
* Do not use more than 25 mg of tissue, as this may cause clogging of the DNA Spin Column and contamination of the purified RNA with genomic DNA.

2. **Add 600 µl Buffer RLT with β-mercaptoethanol, vortex and shake until the tissue is completely lysed and the solution is translucent.**

* Buffer RLT is corrosive, please wear protective equipment when operating.

DNA Purification:

3. **Centrifuge at 13,000 rpm for 2 min, transfer all the supernatant to a DNA Spin Column, close the lid, and centrifuge at 13,000 rpm for 1 min. Transfer the filtrate to a clean RNase-free 1.5 ml centrifuge tube for step 4 operation. Place the DNA Spin Column back into the 2 ml collection tube and proceed to step 6 for parallel DNA/RNA purification.**

* Do not transfer the pellet at the bottom of the tube to avoid clogging the DNA Spin Column.

RNA Purification:

4. **Add 600 µl 70% ethanol to the filtrate and directly pipette 6~8 times with the tip to mix evenly, transfer 600 µl mixture to an RNA Spin Column, close the lid, and centrifuge at 13000 rpm for 1 min. Discard the filtrate, place the RNA Spin Column back into the 2 ml collection tube.**

* If there is a precipitate after mixing with 70% ethanol, please add the precipitate to the Spin Column.

5. **Transfer the remaining mixture to the RNA Spin Column and centrifuge at 13,000 rpm for 1 min. Discard the filtrate and place the RNA Spin Column back into the 2 ml collection tube, which proceeds to step 6 for parallel DNA/RNA purification.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhered to the mouth of the collection tube, you can slap the 2 ml collection tube upside down on a paper towel once.

Parallel DNA/RNA purification:

6. **Add 500 µl Buffer WA to the DNA Spin Column and RNA Spin Column, close the lid, and centrifuge at 13,000 rpm for 1 min.**

* Ensure that absolute ethanol has been added to Buffer WA.

7. **Discard the filtrate, place the DNA Spin Column and the RNA Spin Column back into the 2 ml collection tube, add 600 µl Buffer WBR each, close the lid, and centrifuge at 13,000 rpm for 1 min.**

* Ensure that absolute ethanol has been added to Buffer WBR.

8. **Discard the filtrate, place the DNA Spin Column and the RNA Spin Column back into the 2 ml collection tube, and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.
* Do not omit this step, otherwise the residual ethanol may be mixed in the purified nucleic acid.

9. **Discard the 2 ml collection tube, put the DNA Spin Column and the RNA Spin Column to two clean RNase-free 1.5 ml centrifuge tube. Add 100 µl RNase-Free Water to the DNA Spin Column center, add 50 µl RNase-Free Water to the RNA Spin Column center, incubate at room temperature for 2 min, centrifuge at 12000 rpm for 1 min.**

* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8000 rpm for 1 min to avoid damage to the centrifuge due to the detachment of the lid of the 1.5 ml centrifuge tube.

10. **Discard the Spin Columns, and the eluted DNA or RNA can be immediately used in various molecular biology experiments. Alternatively, store DNA at -20°C and RNA at -70°C for later use.**

* Even if DNA bands are not visible by RNA electrophoresis, the purified RNA should not be considered free of genomic DNA, and if DNA needs to be completely removed, digest the residual DNA with RNase-free DNase I (Simgen Cat. No.8003050).

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Protocol For Cultured Cells

1. **Collect ≤ 1×10^7 cultured cells in a 1.5 ml centrifuge tube, discard the culture medium (culture medium does not need to be fully discarded). Vortex to disperse the cultured cells in the residual micro-culture medium.**

* Do not use too many cells, as this may cause clogging of the Spin Column in subsequent steps.

※Cell collection method:

a) Suspension cultured cells: Centrifuge at 300×g for 5 min to collect about 1×10^7 cultured cells and discard the culture medium.

b) Adherent cultured cells: Collect about 1×10^7 cultured cells by trypsin or cell scraping, centrifuge at 300×g for 5 min, and discard the supernatant.

2. **Add 600 µl Buffer RLT with β-mercaptoethanol, vortex until the cells are completely lysed and the solution is transparent.**

DNA Purification:

3. **Transfer all the lysates of the cells to a DNA Spin Column, close the lid, and centrifuge at 13,000 rpm for 1 min. Transfer the filtrate to a clean RNase-free 1.5 ml centrifuge tube for step 4 operation. Place the DNA Spin Column back into the 2 ml collection tube and proceed to step 6 for parallel DNA/RNA purification.**

RNA Purification:

4. **Add 600 µl 70% ethanol to the filtrate and directly pipette 6~8 times with the tip to mix evenly, transfer 600 µl mixture to an RNA Spin Column, close the lid, and centrifuge at 13000 rpm for 1 min. Discard the filtrate and place the RNA Spin Column back into the 2 ml collection tube.**

* If there is a precipitate after mixing with 70% ethanol, please add the precipitate to the Spin Column.

5. **Transfer the remaining mixture to the RNA Spin Column and centrifuge at 13,000 rpm for 1 min. Discard the filtrate and place the RNA Spin Column back into the 2 ml collection tube, which proceeds to step 6 for parallel DNA/RNA purification.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhered to the mouth of the collection tube, you can slap the 2 ml collection tube upside down on a paper towel once.

Parallel DNA/RNA purification:

6. **Add 500 µl Buffer WA to the DNA Spin Column and RNA Spin Column, close the lid, and centrifuge at 13,000 rpm for 1 min.**

* Ensure that absolute ethanol has been added to Buffer WA.

7. **Discard the filtrate, place the DNA Spin Column and the RNA Spin Column back into the 2 ml collection tube, add 600 µl Buffer WBR each, close the lid, and centrifuge at 13,000 rpm for 1 min.**

* Ensure that absolute ethanol has been added to Buffer WBR.

8. **Discard the filtrate, place the DNA Spin Column and the RNA Spin Column back into the 2 ml collection tube, and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at its full speed for 2 min.

* Do not omit this step, otherwise the residual ethanol may be mixed in the purified nucleic acid.

9. **Discard the 2 ml collection tube, put the DNA Spin Column and the RNA Spin Column to two clean RNase-free 1.5 ml centrifuge tube. Add 100 µl RNase-Free Water to the DNA Spin Column center, add 50 µl RNase-Free Water to the RNA Spin Column center, incubate at room temperature for 2 min, centrifuge at 12000 rpm for 1 min.**

* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8000 rpm for 1 min to avoid damage to the centrifuge due to the detachment of the lid of the 1.5 ml centrifuge tube.

10. **Discard the Spin Column, and the eluted DNA or RNA can be immediately used in various molecular biology experiments. Alternatively, store DNA at -20°C and RNA at -70°C for later use.**

* Even if DNA bands are not visible by RNA electrophoresis, the purified RNA should not be considered free of genomic DNA contamination, and if DNA needs to be completely removed, digest the residual DNA with RNase-free DNase I.